being protected is a high-yield step. Further, all of the protecting groups used should be stable to the usual reaction conditions, such as are used in condensation reactions, to further manipulate the protected compound.

While a protecting group at the 3'-position may desirably be capable of selective cleavage without detrimental effect on a protecting group in the 5'-position, it should be noted that this is not always a necessary feature of the process of the invention. Specifically, in the process of the invention, both the 3'- and the 5'-positions of the dimer intermediates are deprotected, usually simultaneously. Subsequent reaction forms mixtures from which unwanted components are removed.

W can be hydrogen or any one of a wide variety of protecting groups so long as they can be removed independently of the other protective groups (X, Y and Z). Preferably, when W is a protecting group, it is a triphenylmethyl group, such as DMT (dimethoxytrityl) or monomethoxytrityl; a carbonyl-containing group such as FMOC (9-fluorenylmethyloxycarbonyl) or levulinoyl; an acid-cleavable group such as pixyl; a fluoride-cleavable alkylsilyl group such as t-BDMSi (tert-butyl dimethylsilyl), triisopropyl silyl, or trimethylsilyl; and the like. Most preferably, W is the protecting group DMT.

X may be H or any one of a wide variety of protecting groups, so long as it can be removed without destroying the product nucleotide. For example, X may be an alkyl group, such as methyl, ethyl, isopropyl, tert-butyl, or n-hexyl; haloalkyl such as haloethyl; cyanoalkyl such as $-CH_2CH_2CN$; an aryl group such as o-chlorophenyl or methoxyphenyl; and the like. However, most preferably, X is the cyanoalkyl group $-CH_2CH_2CN$.

Y may be H or any one of a wide variety of groups. However, as a guideline to selecting useful groups, Y should preferably be a hydrocarbon. For example, useful Y groups include alkyl groups such as methyl, ethyl, isopropyl, tert-butyl, or n-hexyl. Alternatively, two Y groups, taken together, may form a heterocyclic ring with the nitrogen atom protected, such as morpholino, piperidino, pyrollidino, and the like. Most preferably, Y is an alkyl group, such as methyl or isopropyl.

Z can be any one of large number of different protecting groups and should be chosen so as to have the same characteristics as X. Suitable examples of Z as a protecting group include an alkyl group, such as methyl, ethyl, isopropyl, tert-butyl, or n-hexyl; a cyanoalkyl group such as $-CH_2CH_2CN$; an aryl group such as o-chlorophenyl, or methoxyphenyl; and the like. Preferably, Z is cyanoalkyl such as $-CH_2CH_2CN$.

In a particularly preferred embodiment, W is DMT, X and Z are each $-CH_2CH_2CN$, and Y is an isopropyl group. Methods of manipulating various protective groups with respect to DNA are known to those of ordinary skill in the art. Using similar methods to protect the 2'-hydroxy group of an RNA molecule with a protecting group is also known, e.g., see Wang et al., "Enzymatic and NMR Analysis of Oligoribonucleotides Synthesized with 2'-tert-Butyldimethylsilyl Protected Cyanoethylphosphoramidite Monomers", Nucleic Acids Research, 18:11, 3347-52 (1990).

The bases B^1, B^2 and B^3 are each independently selected from the group consisting of protected adenine, protected guanine, protected cytosine, protected or unprotected thymine and protected or unprotected uracil. When any one or more of B^1, B^2 and B^3 is adenine, cytosine or guanine, it should be protected with a group such as benzoyl, isobutyryl, phenoxyacetyl, methoxyacetyl, an amidine, or the like.

In a particularly preferred embodiment, B^1 is selected from the group consisting of an adenine base protected with a benzoyl protecting group, a thymine base, a cytosine base protected with a benzoyl protecting group, and a guanine base protected with an isobutyryl protecting group. In another preferred embodiment, B^2 is selected from the group consisting of thymine and guanine and, when B^2 is guanine, it is protected with an isobutyryl protecting group.

In yet another preferred embodiment, B^3 is selected from the group consisting of protected adenine and cytosine. When B^3 is either adenine or cytosine, it is preferably protected with a benzoyl protecting group.

Preferred groupings of B^1, B^2 and B^3, with protective groups often preferred for each grouping, are shown below:

$B^3 = A^{bz}$; $B^2 = T$; and $B^1 = A^{bz}$

$B^3 = T$; $B^2 = T$; and $B^1 = C^{bz}$

$B^3 = C^{bz}$; $B^2 = T$; and $B^1 = A^{bz}$

$B^3 = G^{ibu}$; $B^2 = T$; and $B^1 = A^{bz}$

$B^3 = C^{bz}$; $B^2 = G^{ibu}$; and $B^1 = A^{bz}$

"|" in the above formula represents the residue of a ribose or deoxyribose. Ribose is the pentose $CH_2OH(CHOH)_3CHO$ that forms the sugar backbone of an RNA polynucleotide chain in its furanose form by a series of 5'-3' sugar-phosphate links. Deoxyribose has one less hydroxy group at the 2-position of the sugar ring and is the sugar that forms the backbone of a DNA chain.

The compounds of the invention can be made by:

a. treating a nucleoside comprising a base, a ribose or deoxyribose residue, and a first protecting group at the 5' position or the 3' position, with a trialkylsilyl halide to produce the corresponding 3'- and 5'-substituted nucleoside;

b. removing the first protecting group at the 5' or 3' position to produce a 5' or 3' deprotected, 3' or 5'-substituted nucleoside;

c. coupling the 5' or 3' deprotected 3' or 5' substituted nucleoside with a first nucleoside 3' or 5' phosphoramidite and then oxidizing to form a phosphate triester;

d. deprotecting at the 5'- and 3'-termini to give the corresponding 3',5'-dihydroxy dinucleoside;

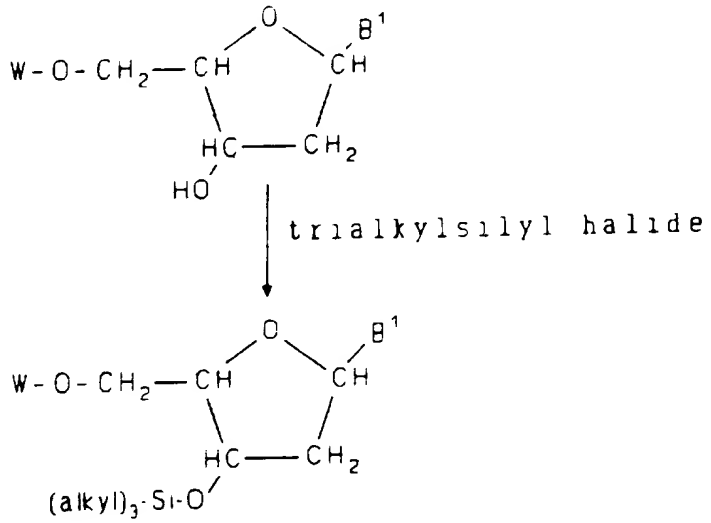
e. coupling the dihydroxy dinucleoside with a second nucleoside 3' or 5' phosphoramidite and oxidizing to produce the two corresponding 3' or 5' hydroxy trinucleotides;

f. separating away unwanted products; and

g. converting the 3'- or 5'-hydroxy trinucleotide to a 3'- or 5' phosphoramidite.

Step "a." of this particularly advantageous process for making the compounds of the invention comprises treating the starting nucleoside, which has a 3'- or 5'-protecting group such

as DMT, with a trialkylsilyl halide, such as t-butyl dimethylsilane halide. For example, when the starting nucleoside has a 5'-protecting group, the product will be a 3'-trialkylsilyl, 5'-protected nucleoside. This reaction is illustrated below:



where W and B_1 are as defined above.

This reaction is typically carried out in the presence of $AgNO_3$ or imidazole, which acts as a catalyst, and an organic solvent, such as pyridine or tetrahydrofuran. Preferably, the reaction takes place in an inert atmosphere, such as that provided by nitrogen or argon gas. The product may be isolated from the rest of the reaction mixture by any convenient method, such as by drowning out in a non-solvent, precipitating out, extraction with an immiscible liquid, evaporation of a solvent, or some combination of these or other methods. A particularly preferred method of adding a 3'-t-butyl dimethylsilyl protecting group is provided by Ogilvie *et al.*, Pure and Appl. Chem., 59, 325-30 (1987).

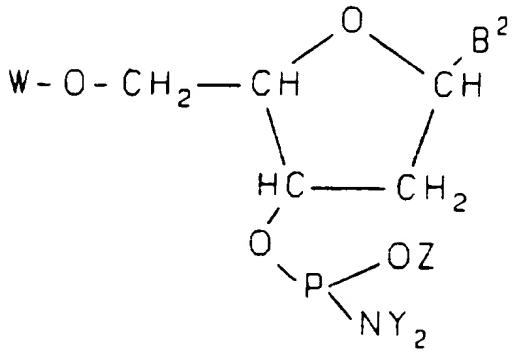
In step "b.", the protecting group at the 5'- or 3'-position can be removed selectively by any one of a number of various procedures, for example, by treatment with a mineral acid, such as HCl or H_2SO_4; an organic acid such as HOAc, dichloroacetic acid, trichloroacetic acid, benzenesulfonic acid; another strong acid; a metal halide such as $ZnBr_2$ or another

Lewis acid; a base such as piperadine or hydrazine; and the like.

In the removing step "b.", a solvent is typically used, such as an alcohol, acetonitrile, diethyl ether, acetone, ethyl acetate, or the like. Preferably, the solvent is acetonitrile.

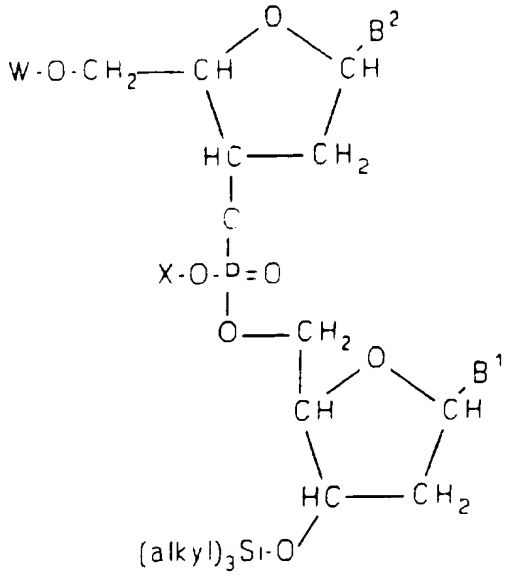
The 3'- or 5'-deprotected product may be isolated from the rest of the reaction mixture by any convenient method, such as by drowning out in a non-solvent, precipitating out, extraction with an immiscible liquid, evaporation of a solvent, or some combination of these or other methods. Preferably, the 3-silyl-protected nucleosides are isolated by crystallization.

Step "c." of the above-described process involves coupling the 5'- or 3'-deprotected, 3'- or 5'-substituted nucleoside with a first nucleoside phosphoramidite, followed by oxidation of the internucleotide trivalent phosphorous to form a phosphate triester. The first nucleoside phosphoramidite has the formula:



where W, Y, Z and B^2 are as defined above. Such phosphoramidites are usually available commercially under the trade name "DNA Amidites." Typically, the nucleoside phosphoramidite is used in the amount of from about 1.0 to about 1.1 equivalents, most preferably about 1.1 equivalents.

After being condensed with, for example, the 5'-deprotected and 3'-substituted nucleoside described above, and after the dinucleotide phosphite triesters have been oxidized to phosphate triesters, the coupled product has the formula:



where W, X, B^1 and B^2 are as defined above.

This dimer formation reaction typically takes place in the presence of a tetrazole for the purpose of activating the phosphoramidite. The amount of tetrazole present can vary widely between about 5 to about 10 equivalents, but preferably is about 10 equivalents. Other materials that can be substituted for tetrazole include benzotriazole and p-nitrophenyl tetrazole.

The dinucleotides are then given a deblocking procedure, such as treatment with acid, to deprotect the 5'- and 3'-termini, using one of the deblocking procedures described above, either alone or in combination with each other or other procedures known to those of ordinary skill in the art. The resulting deprotected 3',5'-dihydroxy dinucleoside is typically isolated from the rest of the reaction mixture by any convenient method, such as by drowning out in a non-solvent, precipitating out, extraction with an immiscible liquid, evaporation of a solvent, or some combination of these or other methods. A preferred isolation method is by chromatography.

Further, when a silyl protecting group is present in step "d.", a silyl cleaving agent such as KF is also typically added to the reaction mixture, primarily to diminish side reactions but also to accelerate the deprotecting reaction.